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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/856,339	08/15/2001	Luet Lok Wong	P02196USO	7723

26271 7590 06/29/2005  
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EXAMINER

PAK, YONG D

ART UNIT	PAPER NUMBER
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1652

DATE MAILED: 06/29/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

<b>Office Action Summary</b>	<b>Application No.</b>	<b>Applicant(s)</b>	
	09/856,339	WONG ET AL.	
	<b>Examiner</b>	<b>Art Unit</b>	
	Yong D. Pak	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

### Status

- 1) ☒ Responsive to communication(s) filed on 16 May 2005.
- 2a) ☐ This action is **FINAL**.                      2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

### Disposition of Claims

- 4) ☒ Claim(s) 2,3,5 and 22-25 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 2,3,5 and 22-25 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

### Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All    b) ☐ Some \*    c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
3. ☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- \* See the attached detailed Office action for a list of the certified copies not received.

### Attachment(s)

- |                                                                                         |                                                                             |
|-----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892)                        | 4) <input type="checkbox"/> Interview Summary (PTO-413)                     |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)    | Paper No(s)/Mail Date. _____                                                |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| Paper No(s)/Mail Date _____                                                             | 6) <input type="checkbox"/> Other: _____                                    |

### **DETAILED ACTION**

This application is a 371 of PCT/GB99/03873.

#### ***Continued Examination Under 37 CFR 1.114***

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on May 16, 2005, canceling claim1, amending claims 2, 5 and 22-24 and adding claim 25, has been entered.

Claims 2-3, 5 and 22-25 are pending and are under consideration.

#### ***Response to Arguments***

Applicant's amendment and arguments filed on May 16, 2005, have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. Specifically, Examiner has withdrawn the previous rejection of claims 2-3, 5 and 22-23 under 35 U.S.C. 112, 1<sup>st</sup> paragraph and a new rejection of claims 2-3, 5 and 22-24 is set forth below.

### ***Claim Objections***

Claim 25 is objected to because of the following informalities: Claim 25 is objected for improper grammar. It appears that a comma is missing after "thereof" in lines 8 and 26. Also, the recitation "at at" should be "of at" in lines 10 and 18. Appropriate correction is required.

Claims 2-3, 5 and 2-25 are objected to because of the recitation of "mutant" and "variant". In order to achieve consistency and uniformity in all the claims, applicants are urged to use either "mutant" or "variant".

Claim 2 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 2 is drawn to a mutant enzyme of P450cam or P450BM-3 and claim 25, from which claim 2 is dependent form, is also drawn to a mutant enzyme of P450cam or P450BM-3. Therefore, claim 2 fails to further limit claim 25.

### ***Claim Rejections - 35 USC § 112***

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

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Claim 25 and claims 2-3 and 22-24 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 25, the phrases "a P450cam variant enzyme corresponding to SEQ ID NO:1" and "a P450BM-3variant enzyme corresponding to SEQ ID NO:24". The metes and bounds of the above phrases are not clear in the context of the claim. The specification does not describe as to how one skilled in the art can determine as to which specific sequence of a given P450cam is a variant "corresponding to SEQ ID NO:1" or a given P450BM-3 is a variant "corresponding to SEQ ID NO:24". Therefore, it is unclear from the specification or from the claims as to what applicants mean by the above phrase. Examiner suggests direct reference to a SEQ ID NO.

Claim 25 and claims 2-3 and 22-24 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 25 recites the phrase "comprising at least two or more mutations at amino acid position which are equivalent to amino acid position... of P450cam enzyme corresponding to SEQ ID NO:1", "comprising at least one or more mutations at amino acid position equivalent to ...of P450BM-3 enzyme corresponding to SEQ ID NO:24". The metes and bounds are not clear in the context of the claims. The specification does not describe as to how one skilled in the art can determine as to which specific amino

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acid in any P450 enzyme is "equivalent" to the amino acid listed. Therefore, it is unclear from the specification or from the claim as to what applicants mean by the above phrase. Furthermore, it will be impossible for the Examiner to do a meaningful search since it is unclear which amino acids are mutated. Examiner suggests direct reference to amino acid positions to clearly define amino acids that are mutated.

Claims 22-23 and 25 and claims 2-3, 5 and 24 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 22-23 and 25 recite the phrase "substituted derivative thereof". The metes and bounds of the structure and function of the substrates are not clear to the Examiner. Literally, the term "derivative" means a substance that can be made from another substance. Therefore, it is not clear to the Examiner either from the specification or from the claims as to what applicants mean by the above phrase. As applicants have not provided a definition for the above phrase, Examiner has interpreted the claims broadly to mean that a "substituted derivative thereof" of pinene, limonene or cyclic sesquiterpenes encompasses molecules which are variants or mutants of a glutathione.

Claim 25 and claims 2-3, 5 and 22-24 depending therefrom are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 25 recites the phrase "homologue variant enzyme". The metes and bounds of the above phrases are not clear in the context of the claim. It is not clear to the Examiner if the claims are drawn to a homologue of an enzyme or a variant of an enzyme.

Claims 3 and 25 and claims 2, 5 and 22-24 depending therefrom rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 3 and 25 recite the phrase "less polar side-chain". The metes and bounds of the above phrases are not clear in the context of the claim. It is not clear to the Examiner which amino acids are considered as having "less polar side-chain" by the applicants. A perusal of the specification did not provide a clear definition for the above phrase. Without a clear definition, those skilled in the art would be unable to conclude if an amino acid has a "less polar side-chain" without knowing the metes and bounds of the phrase.

Claim 5 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 5 recites the phrase "F87A-I395F" and "R47L-Y51F". The metes and bounds of the above phrases are not clear in the context of the claim. It is not clear to the Examiner if the mutant enzyme comprises a range of mutations starting from the amino acid at position 87 through the amino acid position at 395 or if the mutant enzyme comprises double mutations at amino acid positions 87 and 295 or at amino acid positions 47 and 81.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 2-3, 5 and 22-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of oxidizing pinene, limonene or cyclic sesquiterpene using variant of P450BM-3 with SEQ ID NO:24 or a variant of P450Cam with SEQ ID NO:1 comprising an amino acid sequence that has at least 95% sequence identity to SEQ ID NO:1 or 24 and consists of the recited amino acid mutations (amino acid 87, 96, 244, 247 or 248 of P450cam or amino acids 47, 51 or 87 of P450BM-3), does not reasonably provide enablement for a method of oxidizing pinene, limonene, any cyclic sesquiterpene or substituted derivatives of the same using any variant of P450BM0-3 of SEQ ID NO:24 or P450Cam of SEQ ID NO:1. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.



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Factors to be considered in determining whether undue experimentation is required are summarized in In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir. 1988). They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

Claims 2-3, 5 and 22-25 are drawn to a method of oxidizing pinene, limonene or cyclic sesquiterpene using a variant of P450BM0-3 of SEQ ID NO:24 or P450Cam of SEQ ID NO:1 comprising of the recited amino acid mutations. Therefore, these claims are drawn to a P450 variants having any structure, other than having the recited amino acid mutations. The claims are also drawn to a method of oxidizing any substituted derivatives of pinene, limonene or any cyclic sesquiterpene, having any chemical structure and any function.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polypeptides and substituted derivatives of pinene, limonene or any cyclic sesquiterpene encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and

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detailed knowledge of the ways in which the proteins' structure relates to its function.

However, in this case the disclosure is limited to a method of oxidizing pinene, limonene or cyclic sesquiterpene using variant of P450BM-3 of SEQ ID NO:24 or a variant of P450Cam of SEQ ID NO:1, wherein the variant has 95% sequence identity to SEQ ID NO:1 or 24 or consists of the recited amino acid mutations.

It would require undue experimentation of the skilled artisan to make and use the claimed polypeptides. In view of the great breadth of the claim, amount of experimentation required to make the claimed polypeptides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure, the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the polypeptides encompassed by the claims, used in a method of oxidizing pinene, limonene or cyclic sesquiterpene.

While enzyme isolation techniques, recombinant and mutagenesis techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass all modifications and variants of a P450cam of SEQ ID NO:1 or P450BM-3 of SEQ ID NO:24, except the recited amino acid mutations, because the specification does not establish: (A) a universal method to oxidize any substituted derivatives of pinene, limonene and any cyclic sesquiterpenes; (B) regions of the polypeptide structure which may be modified without affecting P450 activity; (C) the general tolerance of P450 to modification and extent of such tolerance; (D) a rational and predictable scheme for modifying any amino acid residue with an expectation of obtaining the desired biological function; (E) a rational and predictable scheme for selecting P450 variants with an expectation of oxidizing any substituted derivatives of pinene, limonene and any cyclic sesquiterpenes; and (F) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including oxidizing any substituted derivatives of pinene, limonene and any cyclic sesquiterpenes using any P450 variants with an enormous number of amino acid modifications of the P450 of SEQ ID NO:1 or 24. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of the P450 variants having catalytic activity is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly,

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extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

### ***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 2-3, 5 and 22-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wong et al.

Claims 2-3, 5 and 22-25 are drawn to a method of oxidizing pinene, limonene or cyclic sesquiterpene or derivatives of said pinene, limonene or cyclic sesquiterpene

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using a variant of P450BM0-3 of SEQ ID NO:24 or P450Cam of SEQ ID NO:1 comprising of the recited amino acid mutations and/or any other amino acid mutations.

Wong et al. (UK Publication GB 2 294 692 A, UK Publication GB 2 306 485 A or U.S. Patent No. 6,117,661) disclose mutant P450cam having mutations at amino acid 87 or 96 of a P450cam (abstract and pages 4-5). The P450cam of Wong et al. and the P450cam of SEQ ID NO:1 of the instant invention are 100% identical (see form PTO-892 - Sequence Alignment). Wong et al. disclose a method of oxidizing various substrates, including monoterpenes and isoprenes, with mutant P450 enzymes of P450<sub>CAM</sub> (pages 19 and 25). Sesquiterpenes and monoterpenes belong to the same family of molecules called terpenes, which contain the signature isoprene units. Since compounds similar in structure will have similar properties, one having ordinary skill in the art would have recognized to use the mutant P450 of Wong et al. to oxidize other terpenes, such as pinene, limonene and longifolene, a sesquiterpene. The mutant mutant of Wong et al. inherently possesses the same material functional characteristics as the mutant P450 claims 2-3, 5 and 22-25 since both mutant P450 enzymes have mutations at amino acids 87 or 96.

Since the Office does not have facilities for examining and comparing applicant's mutant P450 enzyme with the mutant P450 enzyme of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the mutant P450 enzyme of the prior art does not possess the same material functional characteristics of the claimed mutant P450

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enzyme). See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald et al.*, 205 USPQ 594.

Such pinene, limonene and longifolene are commercially available through Sigma Catalog 1995, pages 624, 640 and 837) (form PTO-892).

Therefore, combining the teachings of the above two references, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to oxidize other terpenes, such as pinene, limonene and longifolene using the method taught by Wong et al. One of ordinary skill in the art would have been motivated to oxidize other terpenes instead of chemically synthesizing oxidized products of said substrates. One of ordinary skill in the art would have had a reasonable expectation of success Wong et al. teaches a method of oxidizing terpenes using mutants of P450Cam and since the recited terpenes are commercially available through Sigma Catalog.

Therefore, Wong et al. and Sigma Catalog render claims 2-3, 5 and 22-25 *prima facie* obvious to those skilled in the art.

Claims 1-3, 5 and 22-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Flitsch et al.

Claims 2-3, 5 and 22-25 are drawn to a method of oxidizing pinene, limonene or cyclic sesquiterpene or derivatives of said pinene, limonene or cyclic sesquiterpene using a variant of P450BM0-3 of SEQ ID NO:24 or P450Cam of SEQ ID NO:1 comprising of the recited amino acid mutations and/or any other amino acid mutations.

Flitsch et al. (US Patent disclose mutant P450cam having mutations at amino acid 87 or 96 of a P450cam (abstract and pages 4-5). The P450cam of Flitsch et al. and the P450cam of SEQ ID NO:1 of the instant invention are 100% identical (see form PTO-892 - Sequence Alignment). Flitsch et al. disclose a method of oxidizing various substrates, including monoterpenes and isoprenes, with mutant P450 enzymes of P450<sub>CAM</sub> (pages 19 and 25). Sesquiterpenes and monoterpenes belong to the same family of molecules called terpenes, which contain the signature isoprene units. Since compounds similar in structure will have similar properties, one having ordinary skill in the art would have recognized to use the mutant P450 of Flitsch et al. to oxidize other terpenes, such as pinene, limonene and longifolene, a sesquiterpene. Since compounds similar in structure will have similar properties, one having ordinary skill in the art would have recognized to use the mutant P450 of Wong et al. to oxidize other terpenes, such as pinene, limonene and longifolene, a sesquiterpene. The mutant mutant of Flitsch et al. inherently possesses the same material functional characteristics as the mutant P450 claims 2-3, 5 and 22-25 since both mutant P450 enzymes have mutations at amino acids 87 or 96.

Since the Office does not have facilities for examining and comparing applicant's mutant P450 enzyme with the mutant P450 enzyme of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the mutant P450 enzyme of the prior art does not possess the same material functional characteristics of the claimed mutant P450

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enzyme). See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald et al.*, 205 USPQ 594.

Such pinene, limonene and longifolene are commercially available through Sigma Catalog 1995, pages 624, 640 and 837) (form PTO-892).

Therefore, combining the teachings of the above two references, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to oxidize other terpenes, such as pinene, limonene and longifolene using the method taught by Flitsch et al. One of ordinary skill in the art would have been motivated to oxidize other terpenes instead of chemically synthesizing oxidized products of said substrates. One of ordinary skill in the art would have had a reasonable expectation of success Flitsch et al. teaches a method of oxidizing terpenes using mutants of P450Cam and since the recited terpenes are commercially available through Sigma Catalog.

Therefore, Flitsch et al. and Sigma Catalog render claims 2-3, 5 and 22-25 *prima facie* obvious to those skilled in the art.

In response to the previous Office Action, applicants have traversed the above rejections.

Applicants argue that the oxidation reaction of the claims have surprising, unexpected results because as the declaration discusses that the particular mutant enzymes defined in the claims have an unexpectedly high oxidation activity towards limonene, pinene and cyclic sesquiterpenes. Examiner respectfully disagrees. The



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declaration does not argue that the recited mutant enzymes have an unexpectedly high oxidation activity. Rather, the declaration states that the mutant enzymes comprising multiple mutations have a higher production or oxidation rate, not an unexpectedly higher oxidation rate. Furthermore, those skilled in the art would expect characteristics such as reaction rates to either increase or decrease in mutants.

Applicants also argue that the cited reference do not disclose or suggest mutant P450 having multiple mutations have higher levels of oxidation. However, the claims do not recite any limitations that the mutant enzymes have a higher oxidation activity.

Further, the claims are not solely limited to P450 mutants comprising multiple mutations, but the claims recite using P450 mutants having one mutation and both references use P450 mutants having one mutation.

Applicants also argue that the declaration discusses the structural and physical difference between isoprene and sesquiterpene and explains that if the skilled person knew that isoprene was oxidized by a given enzyme, they would be surprised.

Examiner respectfully disagrees. As applicants have stated, the cited references discuss introducing less polar amino acids into the active site of the P450cam or even amino acids of different size to accommodate large substrates. Also, the cited references use larger molecules than sesquiterpenes as substrates for the P450 mutants (Columns 5-27 of Wong et al. and Columns 5-27 of Flitsch et al.). Therefore, one of ordinary skill in the art would have had a reasonable expectation of oxidizing large substrates, such as large terpenes, like sesquiterpenes. Further, since compounds similar in structure will have similar properties, the ability of the mutant

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P450 enzymes of Wong et al. or Flitsch et al. to act on other terpenes would not have been an unexpected result.

Applicants also argues that the neither Wong et al. nor Flitsch et al. discuss a method of using P450BM-3 mutants. The rejection of claims 2-3, 5 and 22-25 have been amended. However, the claims are drawn to P450cam or P450BM-3 and therefore, the two references teach all embodiments of the claims.

Therefore, it would have been obvious to one having ordinary skill in the art to apply the teachings of Wong et al. or Flitsch et al. to oxidize other terpenes, such as sesquiterpenes.

The declaration under 37 CFR 1.132 filed on September 20, 2004 is insufficient to overcome the rejection of claims 1-3, 5 and 22-24 based upon Wong et al. applied under 35 U.S.C. 103 and Flitsch et al. applied under 35 U.S.C. 103 as set forth in the last Office action because: the declaration fails to overcome the rejections under 35 U.S.C. 103 for the following reasons.

The declaration argues that the references do not discuss oxidation of limonene, pinene or cyclic sesquiterpenes by mutant P450cam monooxygenase with particular combinations of mutations and cites Bell et al. to demonstrate that particular combination of multiple mutations specific in the present claims have a higher production rate and a higher coupling efficiency and give different oxidation products. However, the claims do are not limited to such characteristics of mutant enzymes having a higher oxidation activity or production rate higher or higher coupling efficiency.

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Further, the claims are not solely limited to P450 mutants comprising multiple mutations, but the claims recite using P450 mutants having one mutation and both references use P450 mutants having one mutation.

The declaration also argues that the cited references cannot be used to predict whether or not the substrates recited in the present claims can be oxidized by a mutant P450 enzyme because while sesquiterpenes are structurally related to isoprene and monoterpenes, this does not mean that an enzyme which oxidizes isoprene or a monoterpene would be expected to oxidize a sesquiterpene for two reasons: sesquiterpenes are larger molecules than monoterpenes or isoprene and are much less flexible and adopt a rigid three dimensional structure, requiring the mutant P450cam enzyme having flexible active site to accommodate such large rigid substrate and it would not have been expected that the P450 mutants taught by Wong et al. and Flitsch et al. accommodate these large and rigid sesquiterpenes since the P450 mutants are used to oxidize smaller molecules. Examiner respectfully disagrees. As applicants have stated, the cited references discuss introducing less polar amino acids into the active site of the P450cam or even amino acids of different size to accommodate large substrates. Also, the cited references use larger molecules than sesquiterpenes as substrates for the P450 mutants (Columns 5-27 of Wong et al. and Columns 5-27 of Flitsch et al.). Therefore, one of ordinary skill in the art would have had a reasonable expectation of oxidizing larger terpenes, such as sesquiterpenes. Further, since compounds similar in structure will have similar properties, the ability of the mutant

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P450 enzymes of Wong et al. or Flitsch et al. to act on other terpenes would not have been an unexpected result.

The declaration also argues that the neither Wong et al. nor Flitsch et al. discuss a method of using P450BM-3 mutants. The rejection of claims 2-3, 5 and 22-25 have been amended. However, the claims are drawn to P450cam or P450BM-3 and therefore, the two references teach all embodiments of the claims.

Therefore, it would have been obvious to one having ordinary skill in the art to apply the teachings of Wong et al. or Flitsch et al. to oxidize other terpenes, such as sesquiterpenes.

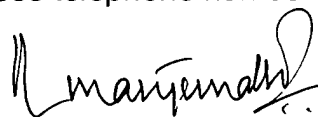
None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Yong D. Pak  
Patent Examiner 1652



Rao Manjunath  
Primary Patent Examiner 1652